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Pharmacokinetics and Disposition of Rilpivirine (TMC278) Nanosuspension as a Long-Acting Injectable Antiretroviral Formulation[▽]

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The next-generation human immunodeficiency virus type 1 (HIV-1) nonnucleoside reverse transcriptase inhibitor rilpivirine (TMC278) was administered in rats and dogs as single intramuscular (IM) or subcutaneous (SC) injections, formulated as a 200-nm nanosuspension. The plasma pharmacokinetics, injection site concentrations, disposition to lymphoid tissues, and tolerability were evaluated in support of its potential use as a once-monthly antiretroviral agent in humans. Rilpivirine plasma concentration-time profiles showed sustained and dose-proportional release over 2 months in rats and over 6 months in dogs. The absolute bioavailability approached 100%, indicating a complete release from the depot, in spite of rilpivirine concentrations still being high at the injection site(s) 3 months after administration in dogs. For both species, IM administration was associated with higher initial peak plasma concentrations and a more rapid washout than SC administration, which resulted in a stable plasma-concentration profile over at least 6 weeks in dogs. The rilpivirine concentrations in the lymph nodes draining the IM injection site exceeded the plasma concentrations by over 100-fold 1 month after administration, while the concentrations in the lymphoid tissues decreased to 3- to 6-fold the plasma concentrations beyond 3 months. These observations suggest uptake of nanoparticles by macrophages, which generates secondary depots in these lymph nodes. Both SC and IM injections were generally well tolerated and safe, with observations of a transient inflammatory response at the injection site. The findings support clinical investigations of rilpivirine nanosuspension as a long-acting formulation to improve adherence during antiretroviral therapy and for preexposure prophylaxis.

The search for improved treatment of human immunodeficiency virus (HIV) infection continues, as the virus not only escapes immunological pressure but also develops resistance against currently available drug therapies. These include nucleoside reverse transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), HIV protease inhibitors (PIs), fusion inhibitors, and the more recent CCR5 entry inhibitors and integrase inhibitors (14, 22). Efficacy has been improved considerably since the introduction of combination antiretroviral therapy (cART), commonly referred to as highly active antiretroviral therapy (HAART), a combination therapy of several anti-HIV agents, usually with different activity profiles (6). Yet, even with long-term use of such combination therapies, the HIV infection is not eradicated, so that chronic treatment is needed in order to keep virus replication suppressed to a level at which the patient remains asymptomatic. Therefore, achieving compliance with medication intake plays a crucial role, which is an even bigger challenge when cART involves a complex drug regimen and high pill burden. Noncompliance has been identified as a critical risk factor for resistance development, further underlining the importance of improving adherence (6, 9, 24, 25). Long-acting formulations of anti-HIV medication that need only infrequent dosing could facilitate maintenance therapy.

As the virus is not eradicated by cART, HIV-infected individuals remain at risk for transmission of the infection to others. To prevent transmission of HIV, a long-acting injectable formulation of antiretroviral medication with low frequency dosing presents major advantages over daily oral therapy for prophylaxis. For example, preexposure prophylaxis may protect newborns and children, who may acquire the virus during labor, delivery, or breastfeeding (mother-to-child transmission) (31). Currently, effective oral antiviral treatment given to the mother at onset of labor and to the baby after the delivery is available, but the risk for subsequent mother-to-child transmission remains present by direct contact and during breastfeeding (5, 15, 16, 20).

In HIV treatment and prophylaxis, ideal candidates for long-acting formulations should interfere early in the HIV life cycle and be effective against a broad range of wild-type and mutant HIV strains with reduced susceptibility to commonly prescribed agents. As NNRTIs interfere early in the cycle of viral replication by preventing the proviral DNA synthesis and integration of the viral genome in the host's DNA, these drugs are good candidates for prophylactic interventions. Yet, first-generation NNRTIs have been associated with rapid emergence of viral resistance, usually with cross-resistance to other NNRTIs (18).

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Rilpivirine (TMC278), a diarylpyrimidine derivative, is a next-generation NNRTI currently in development as an oral formulation for the treatment of HIV, showing an improved resistance profile against NNRTI-resistant HIV-1. *In vitro*, it was shown to have a more pronounced activity against both wild-type and resistant HIV-1 strains, with an increased genetic barrier to viral resistance compared to the first-generation NNRTIs (19). The *in vitro* 50% effective concentration (EC_{50}) for wild-type HIV virus is 0.51 nM (0.18 ng/ml) (10). In a phase IIb study, rilpivirine resulted in durable efficacy and an improved tolerability profile, with fewer neuropsychiatric and metabolic side effects than efavirenz (1, 10, 12, 26). Hence, this drug represents a good candidate for sustained administration, both for treatment and prophylaxis purposes.

Injectable long-acting formulations are currently available in several other therapeutic areas requiring long-term treatment or prophylaxis, such as for psychiatric disorders or contraception (7, 13). The technologies used so far were not suitable for antiviral drugs, as these would require large volumes and considerable amounts of excipients per administration (2). Development of nanosuspensions, however, supports a high drug load per volume, so that long-acting formulations of these poorly water-soluble drugs have come within reach (23). Such nanosuspensions are typically stabilized against aggregation, caking, and precipitation by a surfactant. By altering the size of the nanoparticles, the drug release profile may be tailored. We recently described the development of such nanosuspensions of rilpivirine, making use of its property of being largely insoluble in water and oils (2). A proof-of-concept study showed that rilpivirine nanosuspensions could act as long-acting formulations, releasing rilpivirine up to 3 months after administration. The release of rilpivirine was higher and more stable with a targeted particle size of 200 nm compared to nanosuspensions containing particles of 400 and 800 nm (2).

In the present series of preclinical studies in rats and dogs, the pharmacokinetics and distribution in lymphoid(-like) tissues and injection sites, as well as tolerability, were further characterized after subcutaneous (SC) or intramuscular (IM) administration of long-acting rilpivirine, given as a 200-nm nanosuspension formulation. Doses ranged from 5 mg/kg to approximately 50 mg/kg.

MATERIALS AND METHODS

Ethics. Animals were housed with free access to water all day. Food was accessible all day for rats, while dogs were fed in the morning. Animals were treated in accordance with the provisions for protection of vertebrates that are used for experimental and other scientific purposes and for protection of laboratory animals, per Belgian laws and European conventions (European Council Directives, 1986, and the European Commission's protocol on the protection and welfare of animals used for experimental and other scientific purposes, 2007; http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm). All studies were approved by the local ethics committee on animal experiments and performed in an AAALAC-accredited laboratory, complying with European and Belgian regulations for animal experiments.

Test formulations. A sterile, isotonic nanosuspension consisting of rilpivirine particles with an average diameter of 200 nm, serving as a long-acting formulation, further referred to as rilpivirine long acting (LA), was prepared from the NNRTI rilpivirine (4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]-20 benzonitrile) (TMC278) as previously described, by wet milling of the rilpivirine base, surfactant, and water for injection under aseptic conditions (2). Poloxamer 338 (Pluronics F108), a hydrophilic, nonionic surfactant, was used to enhance solubility and stabilize the colloidal suspension against aggregation. Nanosuspensions were diluted to obtain 25 mg/ml and 100 mg/ml of 200-nm rilpivirine particles and stored at room temperature in closed and labeled containers, protected from light. Concentration, homogeneity, stability, and sterility of these ready-to-use long-acting formulations were checked prior to use, as previously described (2). In the measuring of the volume diameters by Coulter LS230, 10% of the particles were sized below 81 nm, 25% below 120 nm, 50% below 194 nm, 90% below 474 nm, and 95% below 652 nm.

A rilpivirine solution for intravenous (IV) injection was prepared at a 1.25-mg/ml concentration in 75% polyethylene glycol 400/25% (vol/vol) sterile water for injection.

Route of administration. Rilpivirine LA was dosed in rats and dogs by IM injection in the musculus biceps femoris or SC injection in the thoracic region, using a 22G needle at a volume of 0.2 ml/kg body weight in rats (1 site per injection) or dogs (two sites, 0.1 ml/kg/site). One or two days prior to SC or IM dosing, the administration sites were shaven to allow evaluation of skin tolerability. The injection sites were marked immediately after dosing and were kept shaven and marked during the study period to facilitate clinical observation and study of gross pathology.

In studies assessing absolute bioavailability, animals were administered a single IV injection of rilpivirine in polyethylene glycol (PEG) 400/sterile water, as a slow bolus injection with an Acufirm needle at a volume of 1 ml/kg body weight.

Study design. Sprague-Dawley rats weighing from 250 to 350 g and healthy male beagle dogs weighing between 8 and 16 kg were used.

Studies in rats. Different doses and routes of administration were studied in order to evaluate their impact on rilpivirine bioavailability and release profiles over 8 weeks. Rilpivirine LA was administered as a single IM or SC injection at 5 mg/kg and 20 mg/kg body weight or as a single IV injection (solution) at 1.25 mg/kg, the latter to determine the absolute bioavailability after IM and SC administration. Typically, six male rats were used per treatment group for this pharmacokinetic study. Blood samples were drawn at regular times up to 72 h (IV) or 92 h (IM and SC) postdosing and were followed by subsequent weekly samples up to 8 weeks (IM and SC) for determination of rilpivirine concentrations. At necropsy, gross pathological findings were described, and the injection sites were examined histopathologically. Analysis of tissue concentrations of rilpivirine was performed on selected tissues (thymus, spleen, and injection sites).

Studies in dogs. Three studies were performed with beagle dogs. Two studies of male dogs assessed the release profile of rilpivirine from the LA formulation over 6 months after IM or SC administration. The other study was a formal toxicology study of male and female dogs, compliant with the principles of good laboratory practice (GLP), lasting up to 3 months, in order to evaluate the impact of the dose, gender, and route of administration on plasma concentration-time profiles and tissue distribution, as well as the safety and tolerability of rilpivirine LA.

A small-size exploratory study evaluated the basic proof of concept of a long-acting rilpivirine nanosuspension with 200-nm particles (5 mg/kg SC or IM) in 2 dogs per treatment group. Three-month interim results of the plasma pharmacokinetics from this study have been described before (2). Both animals had a biopsy of the iliac and popliteal lymph nodes, 1 month after injection, while one dog per treatment group was sacrificed after 3 and 6 months for a more extensive tissue analysis.

In the second 6-month study, the objective was to study the absolute bioavailability following IM and SC administration. Dogs (six in total) first received an IV dose of rilpivirine solution by slow bolus injection (1 mg/kg, corresponding to 1.25 mg rilpivirine HCl/ml). After a washout period of 3 weeks, rilpivirine LA was administered at a dose of 5 mg/kg, as an IM injection in three out of the six dogs and as an SC injection in the other three dogs. Blood samples were regularly taken up to 3 months and every month thereafter up to 6 months.

In the last two studies, the animals were followed for general tolerability of the long-acting formulation, including clinical observations and body weight, and more specifically for injection site reactions, including ultrasound examination of the administration sites.

In the 3-month toxicology study, male and female dogs were followed for 3 months, following an SC or IM administration of 200 or 400 mg of rilpivirine LA. Six animals were studied per dose, per route of administration, and per gender. Although targeting a single-dose experiment, the volumes for injection were split over two administrations, given 24 h apart, in order not to exceed acceptable daily dosing volumes with the higher dose. The study included the determination of rilpivirine plasma and tissue concentrations, pharmacokinetic dose proportionality, and potential gender effects, as well as clinical observations and histopathology. Three dogs were sacrificed after 1 month, and the other three after 3 months for study of the tissues. Blood samples were drawn frequently up to 92 h after rilpivirine LA administration, and subsequent sampling every week up to 1 or 3 months followed.

Blood and tissue sampling. In rats, 0.3-ml blood samples on EDTA (EDTA Microvette Sarstedt) were taken in alternating fashion from the orbital venous plexus on the following occasions: (i) post-IV dosing of rilpivirine solution, on day 0 at 7 min and 1 h, 7 h, and 48 h in three rats per treatment group and at 20 min, 3 h, 24 h, and 72 h in the three other rats; and (ii) post-SC or -IM dosing of rilpivirine LA, on day 0 at 20 min and 3, 7, and 24 h and further on day 3, day 14, and day 42 at approximately 8 h in three rats per treatment group, and on day 0 at 1 h and 7 h postdose and further on days 8 and 28 at approximately 8 h in the three other rats. Thymus tissue, spleen tissue, and tissue at the site of injection (i.e., muscle after IM injection and skin after SC injection) were collected at necropsy on day 56 (six animals for lymphoid tissues and three animals for the study of the injection site). An additional blood sample was taken at that time from all rats.

In dogs, 2- or 3-ml blood samples on EDTA (EDTA Vacuette Greiner, Greiner Labortechnik N.V.) were taken from the jugular vein. Samples were taken at 20 min and 1, 3, 8, and 24 h postdose on day 0 (after the first administration) and on day 1 (after the second administration). Blood samples were further collected at approximately 8 a.m. on days 8, 15, 22, and 29 from all six dogs of each dosage group and on days 43, 57, 71, and 85 from the last three male and three female dogs of each dosage group. For studies lasting 6 months, after 3 months, one blood sample was taken every month at 8 a.m. An additional blood sample was taken just before sacrifice. After IV administration of the rilpivirine solution, blood samples were also taken at 7 and 15 min after injection. Tissues were sampled at sacrifice and 1 month, 3 months, or 6 months after dosing in the various studies.

Only in the initial exploratory study, biopsy specimens of lymph nodes were taken on day 29, while at necropsy (one dog each after 3 and 6 months of follow-up) tissue samples were taken from the muscle at injection (I) and non-injection (NI) sites and skin (I and NI site), adrenal gland, spleen, thymus, and the iliac, popliteal, axillar, and mandibular lymph nodes.

Blood sample preparation. Within 2 h of blood sampling, samples were centrifuged at room temperature at about 1,500 (rats) or 1,900 (dogs) $\times$ g for 10 min to allow plasma separation. Immediately thereafter, plasma samples were transferred into a second tube and stored in a freezer within 2 h after the start of centrifugation. Tissue samples were stored in a freezer within 2 h of collection.

At all times, blood and plasma samples were protected from light immediately after sampling.

After collection of the last sample, plasma and tissue samples were transferred frozen for analysis.

Tissue preparation. Single-cell suspensions were prepared from thymus and ileac lymph node samples. Half of the tissue material was put in approximately 5 ml Hanks balanced salt solution (HBSS) without Ca^{2+}/Mg^{2+}, buffered with 10 mM HEPES, and supplemented with 0.5 mM EDTA (pH 7.4) at 4°C. Tissues were cut into pieces and homogenized by the use of forceps and a pipette. The crude cell suspension was filtered through a double-layered gauze or over a nylon mesh, 140 to 70 μm, to remove tissue clots, and rinsed with HBSS buffer. The resulting single-cell suspension was centrifuged at 250 $\times$ g for 10 min at 4°C and washed twice with HBBS without Ca^{2+}/Mg^{2+}. The pellet was suspended in 0.5 ml (lymph nodes) or 1 ml (thymus) HBSS, and cell yield and cell viability were determined. Tissue samples and cell preparations were stored in the freezer until further analysis and transferred frozen at approximately −20°C, except for those samples for which only one single-cell suspension was prepared.

Plasma and tissue concentration analysis. Plasma samples, as collected per protocol; tissue samples, collected at biopsy and necropsy; and cell preparations prepared for bioanalysis were analyzed individually for unchanged rilpivirine by means of a liquid chromatography-tandem mass spectrometry (LC/MS-MS) method, validated for rat and dog plasma (28), and developed as a qualified research method for analysis of rilpivirine in tissues. The lower limit of quantification (LLOQ) was 1.0 ng/ml in plasma and 5.0 ng/g in tissues.

Mean plasma concentrations were calculated per treatment group, sampling time, dose, sex, and route of administration. The pharmacokinetic parameters were derived by noncompartmental analysis of averaged plasma rilpivirine concentration-time profiles, using WinNonlin Professional software (version 4.0.1a). Peak plasma concentration (C_{max}), corresponding to peak time (T_{max}) and area under the plasma concentration versus the time curve (AUC) were calculated. In addition, if applicable, the absolute bioavailability (F_{abs}) was estimated relative to intravenously injected rilpivirine solution and was calculated per experiment, based on the AUC values after dose normalization. Dose proportionality and gender differences were evaluated after IM and SC dose administration by descriptive statistics (calculation of the appropriate ratios).

Mean tissue concentrations were calculated and expressed as ng/g tissue; they were evaluated for dose proportionality by descriptive statistics. Tissue-to-
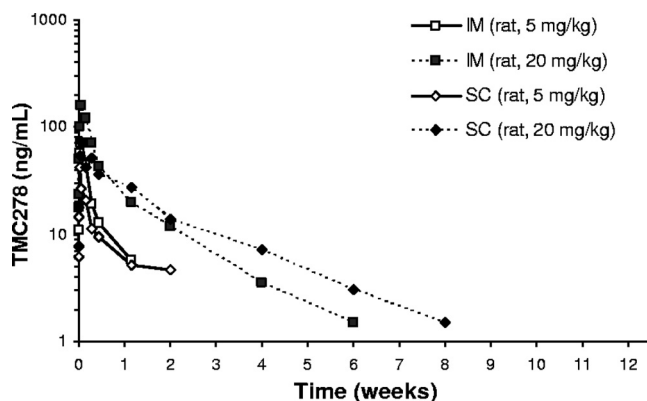

FIG. 1. Mean plasma concentration-time profiles of rilpivirine (TMC278) after a single 5-mg/kg or 20-mg/kg dose of rilpivirine LA in male rats ($n = 6$, alternated sampling per treatment group) showing effect of dose and route of administration.

plasma ratios were not calculated for the rats because the rilpivirine plasma concentrations had fallen below the LLOQ at necropsy.

Tolerability assessment. All animals were observed at least once a day up to day 29, inclusive of signs of waning health, abnormal behavior, or unusual appearance; occurrence of untoward clinical effects, and manifestations of toxic and pharmacological response, moribund state, and mortality. The body weights of the rats and dogs were monitored weekly.

Skin, muscle, and other tissues. Special attention was paid to the injection sites. Methods included (i) gross visual inspection for clinical signs and potential skin reactions at the site of administration; (ii) histology, in which tissues were taken after necropsy, trimmed, embedded, sectioned, and stained with hematoxylin-eosin; and (iii) ultrasonography, which was applied to evaluate *in vivo* injection site reactions. Injection sites (skin and muscle) were visualized with a 10.0-MHz linear array, on day 0 (4 h after dosing), day 1 (SC and IM sites), and day 3 and weekly afterward up to day 27 inclusively (IM sites). The SC-injected animals were examined only up to 1 day after the injection due to minimal reactions at the administration sites, rendering this examination of limited relevance.

RESULTS

Pharmacokinetic profiles. After a single IM or SC administration of rilpivirine LA in male rats, rilpivirine plasma concentrations increased gradually up to 7 h postdose and declined thereafter, remaining detectable up to 8 (IM) to 14 (SC) days postdose at 5 mg/kg, and 42 (IM and SC) days postdose at 20 mg/kg (Fig. 1). The 6-month studies in dogs showed sustained plasma concentrations over time, with levels going below 1 ng/ml only 3 months after IM administration, while progressively further decreasing from 2 to 3 ng/ml at 3 months to 1 ng/ml or below the LLOQ at 6 months after SC administration (Fig. 2). In the 3-month study assessing the 200- and 400-mg doses of rilpivirine LA in male and female dogs (Fig. 3), IM plasma concentration-time curves for both sexes were characterized by distinct initial peaks above 2,000 ng/ml, reached between 0.33 h and 1 h after the first or second dose; plasma concentrations declined fast over the first 2 days and more gradually afterwards until day 85 postdose. With SC dosing, rilpivirine peak plasma levels were lower, and plasma levels remained more sustained over the duration of the 3-month study.

IM versus SC administration. (i) Rats. The decline in rilpivirine plasma levels was slightly faster after IM compared to SC administration (Fig. 1). The C_{max}s were approximately 2-fold higher after IM than after SC administration of equiv-
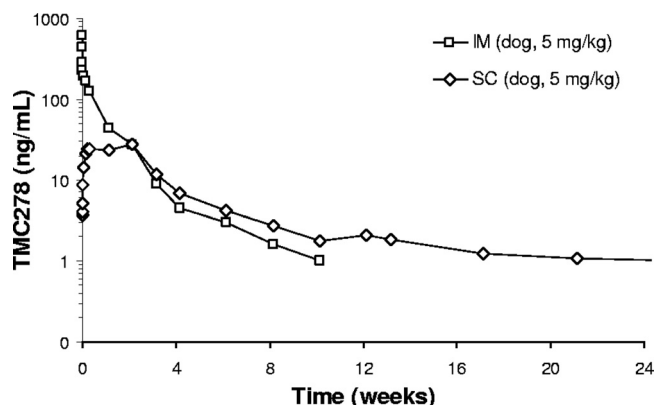
FIG. 2. Six-month mean plasma concentration-time profiles of rilpivirine (TMC278) after IM and SC administration of a single 5-mg/kg dose of rilpivirine LA in male dogs (n = 3 per treatment group; LLOQ = 1 ng/ml).

alent dose levels, but the AUC from 0 to infinity (AUC_{0-inf}) values were comparable for the 2 dosing routes (Table 1).

(ii) Dogs. In all three studies, IM administration was associated with a higher initial release of rilpivirine and faster clearance over the 3- or 6-month observation period compared with SC administration, which resulted in a more stable, sustained release plasma concentration profile (Fig. 2 and 3; Table 1). In the 200- to 400-mg dosing study of male and female dogs (Fig. 3), IM administration was followed by clear superimposed spikes within 1 h from administration of the first or second IM dose administration, while this initial release was not observed after SC dosing; a rapid increase in plasma concentrations occurred over 24 to 48 h and was followed by an essentially flat plasma concentration-time profile for 1 to 3 months. At equivalent doses, the exposure to rilpivirine (both C_{max} and AUC) was higher after IM than after SC dosing.

Dose proportionality. (i) Rats. The C_{max} showed a less-than-dose-proportional increase (Table 1): C_{max}s were 70.6 and 158 ng/ml after IM administration and 42.0 and 72.9 ng/ml after SC administration at 5 and 20 mg/kg, respectively. The increase in the AUC was essentially dose proportional, with a 4.0- and 4.4-fold increase in the AUC from 0 to 56 days (AUC_{0-56d}) after IM and SC administration, respectively, for a 4-fold increase in dose (AUC_{0-56d}, 3.8 and 15.3 μg · h/ml [IM] and 3.5 and 15.5 μg · h/ml [SC] at 5 and 20 mg/kg).

(ii) Dogs. In the 200- to 400-mg dose study, plasma pharmacokinetics were essentially dose-proportional after SC administration with 1.9- to 2.2-fold increases in the C_{max} and AUC for a 2-fold dose increase; mean C_{max}s after a 200-mg and 400-mg dose were 107 and 222 ng/ml in males and 191 and 225 ng/ml in females, and corresponding AUC_{0-85d} values 97.8 and 219 μg · h/ml and 134 and 234 μg · h/ml, respectively. After 3 months, plasma concentrations were 11.8 and 37.0 ng/ml in males and 21.3 and 32.8 ng/ml in females, respectively.

After IM administration of the 200- and 400-mg doses, mean C_{max}s were 2,130 and 2,170 ng/ml in males and 1,440 and 5,260 ng/ml in females, respectively. The AUC_{0-85d} values were 148 and 281 μg · h/ml in males and 121 and 265 μg · h/ml in females, the ratios varying between 1.7 and 2.2 for a 2-fold dose increase.

Gender. There were no major differences between male and female dogs in pharmacokinetics. The female/male ratio for the C_{max} was 0.7 to 0.8 for 200 mg and 0.9 to 2.4 for 400 mg.

Absolute bioavailability. (i) Rats. The AUC_{0-inf} for the rilpivirine solution after a single IV administration reached 1,230 ng · h/ml. The absolute bioavailability of rilpivirine LA, calculated as per day 56, ranged between 70 and 80% after IM and SC administration (Table 1).

(ii) Dogs (6 months). The AUC_{0-inf} was 6,210 ng · h/ml after IV administration of the rilpivirine solution. The absolute bioavailability after IM and SC administration of rilpivirine LA, determined at 3 months postdose, was 102% and 80%, respectively (Table 1).

Tissue concentrations at site of injection. (i) Rats. With 5- and 20-mg/kg injections of rilpivirine LA, rilpivirine concentrations at the injection site at necropsy (8 weeks) were 1,980
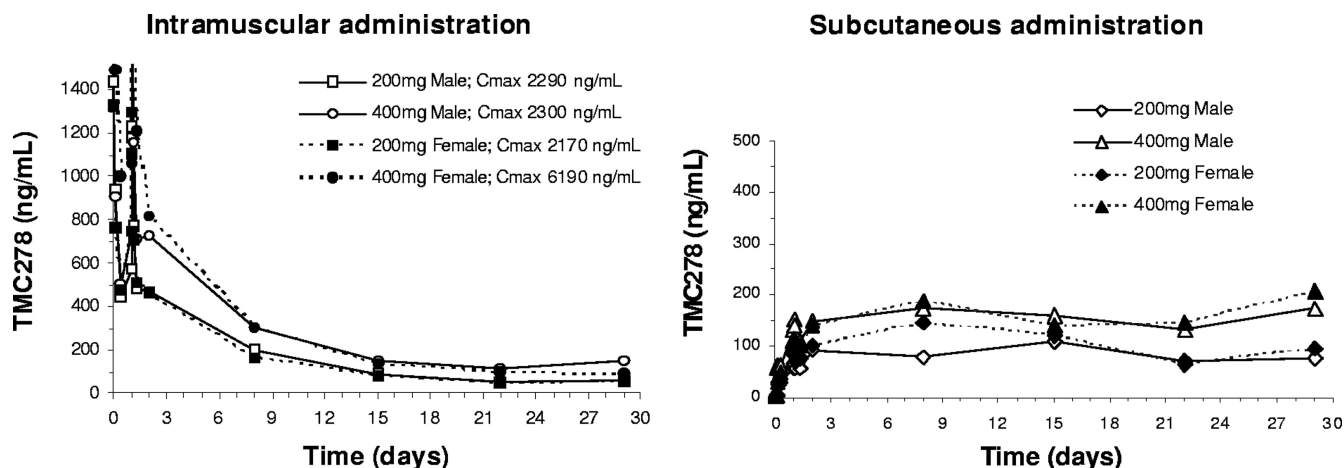
FIG. 3. Mean plasma concentration-time profiles of rilpivirine (TMC278) after administration as LA injection showing effect of dose (200 to 400 mg), route of administration, and gender on single-dose pharmacokinetics of rilpivirine LA during 1 month of follow-up (n = 3 per dosage and gender). Because of restrictions on the volume that can be injected at once in a dog, the 200-mg dose was administered as two single injections (1 site), and the 400 mg as two double injections (2 skin or muscle sites), 24 h apart.

TABLE 1. Pharmacokinetics after administration of a formulation of rilpivirine LA (200-nm nanosuspension) in rats and dogs[c]

No. and gender of indicated species/group[a]	Total study duration	Route	Dose	Interval postdose (days)	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-last} (ng · h/ml)	F_{abs} (%)	Dose proportionality (AUC ratio vs lower dose)
Rat		IV[b]	1.25 mg/kg	2			1,230[d]	100	
6 M	8 wk	IM	5 mg/kg	56	71	7	3,840	78	
		IM	20 mg/kg	56	158	7	15,300	78	4.0
		SC	5 mg/kg	56	42	3	3,540	72	
		SC	20 mg/kg	56	73	7	15,500	78	4.4
Dog, study 1									
6 M	3 mo	IM	200 mg	29	2,290	0.33	117,000	ND	
		IM	400 mg	29	2,300	17	188,000		
6 F		IM	200 mg	29	2,170	4.6	109,000		
		IM	400 mg	29	6,190	8.3	204,000		
6 M		SC	200 mg	29	119	280	5,6800		
		SC	200 mg	29	210	440	105,000		
6 F		SC	400 mg	29	185	220	70,600		
		SC	400 mg	29	220	450	109,000		
3 M		IM	200 mg	85	2,130	0.33	148,000	ND	
		IM	400 mg	85	2,170	17	281,000		2.2
3 F		IM	200 mg	85	1,140	0.55	121,000		
		IM	400 mg	85	5,260	16	265,000		1.7
3 M		SC	200 mg	85	107	250	97,800		
		SC	400 mg	85	222	480	219,000		1.9
3 F		SC	200 mg	85	191	360	134,000		
		SC	400 mg	85	225	300	234,000		2.2
Dog, study 2		IV[b]	1.25 mg/kg	2			8,661[d]	100	
3 M	6 mo	IM	5 mg/kg	176	619	0.5	23,200	102	
		SC	5 mg/kg	176	31.4	288	19,700	80	
Dog, study 3		IM	5 mg/kg	184	173	24	39,400	ND	
1 M	6 mo	SC	5 mg/kg	184	38	144	24,400	ND	

[a] M, male; F, female.
[b] IV solution was administered to 6 rats and dogs, and the AUC_{0-inf} was taken as 100% absolute bioavailability; in the 3-month formal toxicity study, the number of animals was halved after 1 month in order to allow tissue analysis after necropsy.
[c] IV studies in rats and dogs presented to calculate F_{abs}. IV, intravenous; IM, intramuscular; SC, subcutaneous administration; ND, not determined.
[d] AUC_{0-inf}.

ng/g and 8,340 ng/g in muscle after IM administration and <8.19 ng/g (median value) and 102,000 ng/g in skin after SC administration, respectively (Table 2).

(ii) Dogs. As illustrated by the rilpivirine concentrations in plasma, muscle, and skin samples 3 and 6 months postdose in the dogs (Table 2), these were the highest at the injection site, being the muscle in the case of IM administration and the skin in the case of SC administration. Concentrations were much lower in the same tissue from noninjection sites. In line with the decline in plasma levels, the rilpivirine

TABLE 2. Tissue distribution of unchanged rilpivirine (ng/mg) in plasma samples and at site of injection after IM or SC single-dose administration of rilpivirine LA in rats and dogs

No. and gender of indicated species/group	Route	Dose (mg/kg)	Interval postdose	Mean concn of rilpivirine[b]				
				C_{plasma} (ng/ml)	I vs NI sites (ng/g)			
					$C_{muscle\ I}$	$C_{muscle\ NI}$	$C_{skin\ I}$	$C_{skin\ NI}$
Rat, 3 M	IM	5	56 days	<4*	1,980			
		20	56 days	<2*	8,340[c]			
	SC	5	56 days	<2*			8.2*	
		20	56 days	<2*			102,000	
Dog, 1 M[a]	IM	5	3 months	<1[d]	142,000	84.1	16.7	12.4
			6 months	<1[d]	11,500	<5.0[d]	9.5	6.7
	SC	5	3 months	3.3	467	18.5	223,000	24.2
			6 months	1.3	48	<5.0[d]	143,000	13.3

[a] Only 1 dog per group sacrificed for ethical reasons.
[b] *, median values; I, injection; NI, noninjection.
[c] $n = 2$.
[d] LLOQ in plasma = 1 ng/ml and in tissue = 5 ng/g.

TABLE 3. Concentrations, tissue-to-plasma ratios, and dose proportionality of unchanged rilpivirine in plasma, spleen, and thymus samples 1 and 3 months after IM or SC administration of a 200- or 400-mg dose of rilpivirine LA in male and female dogs[a]

Route	Dose (mg)	Rilpivirine concn 1 mo postdose						Rilpivirine concn 3 mo postdose					
		Males (n = 3)			Females (n = 3)			Males (n = 3)			Females (n = 3)		
		Plasma (ng/ml)	Spleen (ng/g)	Thymus (ng/g)	Plasma (ng/ml)	Spleen (ng/g)	Thymus (ng/g)	Plasma (ng/ml)	Spleen (ng/g)	Thymus (ng/g)	Plasma (ng/ml)	Spleen (ng/g)	Thymus (ng/g)
SC	200	95	189 (2.0)	576 (6.0)	71.8	14.5 (2.0)	536 (3.9)	15.2	36.2 (2.5)	84.2 (5.9)	26.8	49.5 (1.9)	105 (4.0)
	400	209	569 (2.9)	606 (2.9)	227	285 (2.5)	1204 (5.0)	48.4	11.3 (2.3)	173 (3.5)	36.0	67.5 (1.9)	101 (2.9)
Dose proportionality		2.2	3.0	1.1	1.9	3.7	4.2	3.1	3.1	2.1	1.3	1.9	2.9
IM	200	74.5	133 (1.8)	234 (3.2)	61	142 (2.3)	353 (5.7)	9.2	23.8 (2.7)	34.4 (3.8)	10.1	22.1 (2.2)	27.4 (2.8)
	400	158	347 (2.2)	634 (4.0)	118	311 (2.6)	676 (6.0)	22.1	63.5 (2.9)	76.0 (3.9)	20.3	46.1 (2.3)	109 (4.9)
Dose proportionality		3.2	2.6	2.7	1.9	2.2	1.9	2.4	2.7	2.2	2.0	2.1	4.0

[a] Values in parentheses indicate tissue-to-plasma ratios.

concentrations at the injection site declined faster from month 3 to month 6 in the injected muscle than the skin.

Tissue concentrations in other tissues. (i) Rats. The rilpivirine concentrations at necropsy (8 weeks after administration) were below 2.00 or 4.00 ng/ml in plasma (median) and below the LLOQ (<5.00 ng/g) in thymus and spleen for the different doses or routes of administration.

(ii) Dogs. Three months after SC and IM administration of a 200- or 400-mg dose, the rilpivirine concentrations in brain (not shown) and spleen (Table 3) tissue were about two times higher than those in plasma samples. The highest tissue-to-plasma ratios were observed with the thymus, reaching values in males and females varying between 3 and 6, both after SC and IM administration and at both observation points (1 and 3 months after administration) (Table 3). No apparent gender differences were observed, and, in general, the tissue concentrations decreased at a rate similar to the decline in concentrations in the plasma samples. Tissue concentrations were essentially dose proportional for both genders.

In the 6-month dog study, tissue analysis of lymph nodes taken by biopsy showed very high concentrations of rilpivirine in draining lymph nodes adjacent to the injection site after IM administration. In contrast, the concentrations in these lymph nodes after SC administration and in peripheral lymph nodes for both routes of administration were only slightly elevated. All lymph node concentrations decreased after 3 and 6 months (Table 4).

Tolerability. Overall, rilpivirine LA was well tolerated, both in rats and dogs. Gain in body weight was normal. Rilpivirine

LA was also generally well tolerated at the site of injection. In rats, a transient swelling at the site of the injection was noted for 4 out of 6 rats between days 4 and 19 after SC administration of the 20-mg/kg dose. With dogs, no major untoward effects were seen at the site of injection, based on gross visual inspection. A slight, palpable, and transient swelling at 8 out of 36 injection sites (in 12 dogs) was observed between 2 and 4 weeks after SC administration. At 4 out of the 36 IM injection sites, some hardening was observed in the 3-month formal tolerability study, and none in the 6-month studies. At histological examination, white deposits were observed with most animals (granulomatous after SC administration; multifocal after IM administration), decreasing over time, with no or minimal inflammatory signs after 3 months. Ultrasound evaluation revealed echoes in an ordered fashion according to the longitudinal direction of the muscle fibers after IM administration, while cloudy, more echogenic areas were observed after SC dosing. With these techniques, there was no evidence of irritation, abscess formation, foreign-body reaction due to encapsulation of deposits, or macrophage giant-cell infiltration.

DISCUSSION

These experiments in rats and dogs showed that a 200-nm nanosuspension of rilpivirine can function as a long-acting injection, releasing rilpivirine for at least 2 and up to 6 months. Despite differences between dogs and rats, the pharmacokinetic profiles showed sustained exposures to rilpivirine.

Plasma and tissue exposure following rilpivirine LA admin-

TABLE 4. Concentrations of unchanged rilpivirine at 1, 3 and 6 months after IM or SC single-dose administration of rilpivirine LA (5mg/kg) in plasma, liver, lymph nodes, and lymphoid tissues of dogs[a]

Route	Interval postdose (mo)	Concn							
		C_{plasma} (ng/ml)	C_{liver} (ng/g)	Lymph nodes				Lymphoid tissues	
				C_{iliac} (ng/g)	$C_{popliteal}$ (ng/g)	$C_{axillar}$ (ng/g)	$C_{mandibular}$ (ng/g)	C_{spleen} (ng/g)	C_{thymus} (ng/g)
IM	1	11.3		19,035 (168)	6,495 (574)				
	3	<LLOQ	20.8	15.3	13.7	25.5	10.0	<5.0	14.7
	6	<LLOQ	11.6	10.7	30.7	12.2	6.9	<5.0	7.2
SC	1	16.8		64.9 (3.9)	68.2 (4.1)				
	3	3.3	45.6 (14)	16.9 (5.2)	26.8 (8.2)	22.1 (6.8)	13.1 (4.0)	9.7 (3.0)	43.6 (13)
	6	1.3	31.1 (24)	9.9 (7.7)	5.5 (4.3)	8.6 (6.7)	7.8 (6.1)	7.1 (5.5)	20.2 (16)

[a] n = 2 per treatment group for the 1-month biopsy samples taken from the iliac and popliteal nodes. n = 1 sacrificed per treatment group at 3 and 6 months for ethical reasons. LLOQ < 1.00 ng/ml. Numbers in parentheses indicate tissue-to-plasma ratios. For the tissue distribution at the sites of injection, see Table 3.
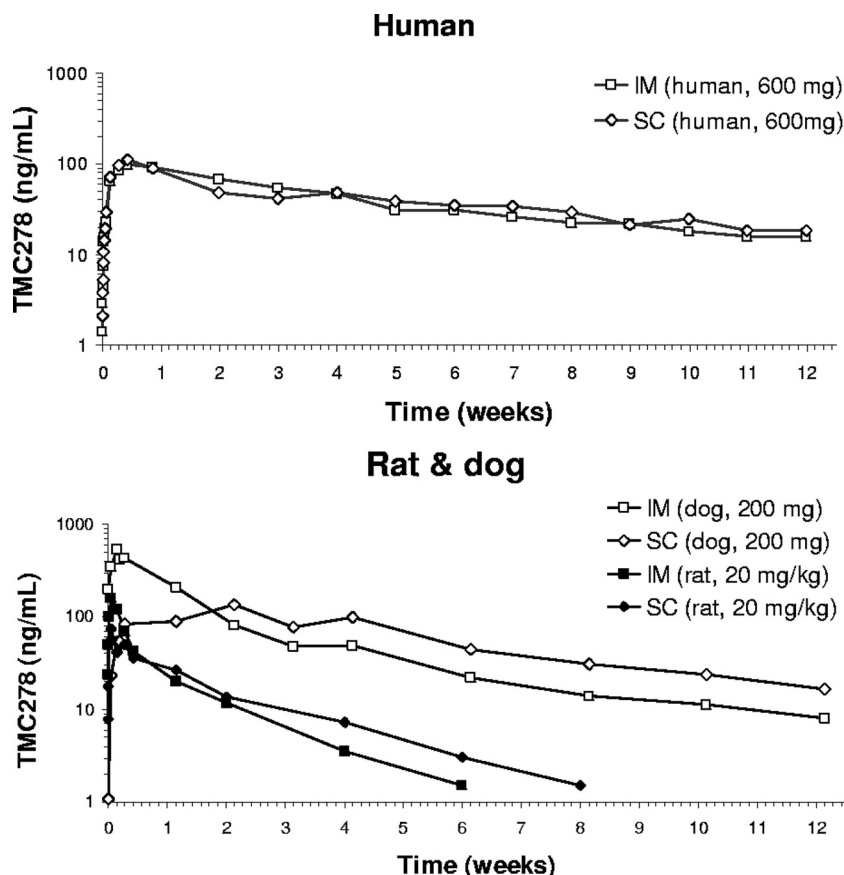
FIG. 4. Species comparison of rilpivirine (TMC278) plasma concentration-time profiles after single SC or IM administration of rilpivirine LA, showing (i) higher clearance rates in rats but similar rates in dogs and humans and (ii) a difference in initial plasma concentration-time profile between IM and SC dosings in dogs and, to a lesser extent, in rats but no differences in humans. (Mean data from male rats [$n = 3$], male and female dogs [$n = 6$ in total] [current studies], and humans [$n = 6$] [29]).

istration were essentially dose proportional over the tested dose range. The absolute bioavailability was 70 to 80% after 8 weeks in rats and 80 to 100% after 6 months in dogs. Although rilpivirine concentrations at the injection sites remained high compared to those at the noninjection sites, the observed absolute bioavailability suggests that the compound was largely released from the injection sites within 8 weeks and 6 months after dosing in rats and dogs, respectively. Also, following SC injection, the progressively decreasing plasma concentration-time profile for dogs (from 2 or 3 ng/ml at 3 months to 1 ng/ml or below the LLOQ at 6 months) supported predictions of a complete release of rilpivirine and of a washout at 1 year postdosing.

In line with the preliminary observations with dogs (2), plasma concentration-time profiles for dogs—and for rats—were more stable after SC administration than after IM administration. However, the IM route was associated with faster initial release of rilpivirine in dogs, as reflected by the higher C_{max} and lower T_{max}. On top of the peak in the plasma concentration-time profiles, spikes (short-lived higher plasma concentrations) were observed immediately after IM administration of the nanosuspension. This may potentially be due to the smaller nanoparticles entering directly into the circulation following injection, as nanoparticles up to 100 nm may readily

cross membranes (17). This is more likely to occur after IM injection, due to the higher vascularization of the muscle structure and the somewhat higher pressure on the injected volume, than after SC injections. Such spikes, however, were not observed with the rats, for which plasma concentrations after reaching C_{max} decreased somewhat faster after IM than after SC administration.

In a recent human pharmacokinetic study of a single dose of the 200-nm long-acting rilpivirine nanosuspension, no relevant differences between IM and SC dosing were observed (29). Species differences in pharmacokinetic profiles are depicted in Fig. 4. This figure also shows the (apparently) similar clearance rates of rilpivirine LA for dogs and humans. This suggests that the pronounced differences between IM and SC administrations observed with dogs for initial release from a 200-nm long-acting nanosuspension may be species specific. A plausible explanation could be the difference in muscle structures between species, dogs having softer and more vascularized striated muscles than rats and humans, allowing greater spreading and faster distribution toward the blood circulation after IM administration. Observations during ultrasound examination and macroscopic inspection of injection sites in our laboratory have revealed that the spreading of an injectable is greater in dog muscle than in the muscles of rats. Better

spreading implies a larger surface area contact with the surrounding tissue, which, together with the better vascularization, will result in a faster distribution. This spreading effect in the muscle will also be more apparent at lower than at higher concentrations, which is supported by the T_{max} values of rilpivirine LA for the dog ($T_{max} = 0.33$ to 4.6 h for the 200-mg dose, $T_{max} = 8$ to 17 h for the 400-mg dose). In rats, however, IM injections are often not truly IM, but rather between the muscles (intermuscular) due to their small muscles and may therefore become more comparable with SC injections. Also, rat muscles are less vascularized and harder, with less endo- and perimysium (connective tissue surrounding muscle fibers), which decreases the spreading and reduces the surface area contact within the muscle, explaining the lower impact of route of administration than that observed with the dog. The overall faster clearance of rilpivirine from muscles or skin in rats can be explained by rats having a higher metabolic rate than dogs.

In humans, spreading will also depend on the muscle in which the formulation is injected (e.g., the deltoid versus the gluteus maximus). Spreading will be higher in active muscles (more friction between muscle fibers) than in less-active muscles (3). After SC injection, the spreading of the formulation will be more limited and associated with less friction of a smaller surface area contact.

Rilpivirine administered as an LA injectable appeared to be well tolerated in rats and dogs. In a study of the injection site, only a minimal inflammation, typical for administration of injections, was observed. Although concentrations of rilpivirine were high at the site of injection after up to 3 months, this did not induce any particular signs of toxicity. As discussed above, the bioavailability close to 100% indicates a complete release from the injection site, and, therefore, no risk of remaining deposits is anticipated. The observed (very) high concentrations in lymph nodes did not induce signs of local toxicity (detailed results not shown), suggesting that rilpivirine is locally present as undissolved (nano)particles rather than dissolved drug.

Also, from a therapeutic perspective, the highly elevated rilpivirine tissue concentration in lymph nodes and lymphoid tissues in dogs is an interesting observation. At 1 month after administration, rilpivirine tissue concentrations in the draining lymph nodes adjacent to the IM injection site exceeded plasma concentrations by over 100-fold, while at later time points, concentrations in lymph nodes and lymphoid tissue (spleen and thymus) were 3- to 6-fold higher than in plasma. On histological inspection, activated macrophages with vacuolated eosinophilic content were observed. These findings suggest a role for macrophages in the uptake and transport of the rilpivirine nanoparticles from the injection site, thereby generating secondary depots in these lymph nodes, as previously reported for other drugs (23). These very high concentrations of rilpivirine in draining lymph nodes adjacent to the injection site 1 month after IM administration of rilpivirine LA were not observed to the same extent after SC administration. It is assumed that this is related to the higher blood perfusion of the muscle than of the skin or subcutaneous tissue. Macrophages apparently act as carriers that can deliver the drug to the lymphoid tissues, which subsequently function as secondary drug reservoirs. These findings are interesting, as the lymphatic system is a key reservoir of HIV virus with active viral replication. In the early phase of the acute viral syndrome, the HIV virus is found particularly in virus-expressing cells in the lymph nodes, and, throughout the period of clinical latency, the HIV virus is active in lymphoid tissue, even at times when minimal viral activity is demonstrated in blood (21). Hence, secondary long-acting drug reservoirs and elevated drug concentrations in lymph nodes may add to the antiviral activity of rilpivirine. This concept deserves further study in the future.

These pharmacokinetic studies in rats and dogs have supported the first clinical trial of rilpivirine LA with human healthy volunteers. Suspensions of 200-nm nanoparticles allow injection of rilpivirine loads in a dense fashion and therefore deliver adequate doses in small volumes for injection to produce therapeutic plasma concentrations. Recently, a single-dose pharmacokinetic study of humans was presented, showing sustained rilpivirine plasma concentrations that fell below 10 ng/ml only by 3 to 6 months after IM or SC administration of a 200- to 600-mg dose (29, 30). The IM route appeared to be better tolerated than the SC route. Pharmacokinetic simulations demonstrated that once-monthly SC or IM administrations of rilpivirine LA in humans will maintain plasma concentrations above the mean minimal plasma concentrations of 73 to 95 ng/ml observed after oral once-daily dosing of 25 mg rilpivirine (1, 12). This oral regimen has been shown to be as efficacious as efavirenz in phase II trials (10). By showing similarly sustained plasma exposure, as shown here with dogs, this study supports the further clinical evaluation of parenteral nanosuspensions of poorly water-soluble anti-HIV drugs for therapeutic and prophylactic use of HIV inhibitors with an injection formulation that needs infrequent administration.

Several challenges remain for clinical application of long-acting injectables. A long-lasting presence and release of drug may be a concern in the case of adverse effects. While the tolerability of oral rilpivirine to date is good and has been shown to be superior to those of its competitors (10), an initial treatment with oral rilpivirine followed by maintenance with rilpivirine LA may establish the absence of individual intolerabilities. Also, for a full low-frequency parenteral cART, two more suitable antiretrovirals would ideally be given in one injection. For maintenance of viral suppression or prophylaxis, a single highly potent antiretroviral, such as rilpivirine, may be considered, but clearly this is not an accepted treatment paradigm yet. Also, low-level exposure during the long-lasting washout at the end of therapy with long-acting antiretrovirals poses a potential risk for induction of viral resistance; these may need to be countered by an early changeover to an alternative, likely oral cART.

The potential advantages of the concept are obvious: infrequent parenteral administration of antiretrovirals may help to improve treatment compliance, reduce gastrointestinal side effects, and eliminate the impact of food on bioavailability (seen with oral rilpivirine [26] and other anti-HIV drugs). For a dose regimen of, e.g., once monthly, there is conceivably a 1-week recall period, which also makes guided treatment compliance practically more feasible. Other benefits of sustaining therapeutic plasma concentrations for long periods of time, as part of cART, could be to reduce the resistance and rebound of viral replication (4, 8, 11, 27, 32), as well as prophylaxis against HIV transmission.

In conclusion, sustained plasma concentration-time profiles

were obtained for several months for rats and dogs after a single dose of a nanosuspension of rilpivirine (200-nm particle size). These findings with animals support further clinical evaluation of the long-acting formulation for improving compliance in HIV treatment, as well as for prophylaxis against HIV transmission.

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